In Vivo Antitumor Activity of SU11248, a Novel Tyrosine Kinase Inhibitor Targeting Vascular Endothelial Growth Factor and Platelet-derived Growth Factor Receptors: Determination of a Pharmacokinetic/Pharmacodynamic Relationship

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ABSTRACT

One challenging aspect in the clinical development of molecularly targeted therapies, which represent a new and promising approach to treating cancers, has been the identification of a biologically active dose rather than a maximum tolerated dose. The goal of the present study was to identify a pharmacokinetic/pharmacodynamic relationship in preclinical models that could be used to help guide selection of a clinical dose. SU11248, a novel small molecule receptor tyrosine kinase inhibitor with direct antitumor as well as antiangiogenic activity via targeting the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), KIT, and FLT3 receptor tyrosine kinases, was used as the pharmacological agent in these studies. In mouse xenograft models, SU11248 exhibited broad and potent antitumor activity causing regression, growth arrest, or substantially reduced growth of various established xenografts derived from human or rat tumor cell lines. To predict the target SU11248 exposure required to achieve antitumor activity in mouse xenograft models, we directly measured target phosphorylation in tumor xenografts before and after SU11248 treatment and correlated this with plasma inhibitor levels. In target modulation studies *in vivo*, SU11248 selectively inhibited Flk-1/KDR (VEGF receptor 2) and PDGF receptor β phosphorylation (in a time- and dose-dependent manner) when plasma concentrations of inhibitor reached or exceeded 50–100 ng/ml. Similar results were obtained in a functional assay of VEGF-induced vascular permeability *in vivo*. Constant inhibition of VEGFR2 and PDGF receptor β phosphorylation was not required for efficacy; at highly efficacious doses, inhibition was sustained for 12 h of a 24-h dosing interval. The pharmacokinetic/pharmacodynamic relationship established for SU11248 in these preclinical studies has aided in the design, selection, and evaluation of dosing regimens being tested in human trials.

INTRODUCTION

The receptor tyrosine kinases (RTKs)[2] are transmembrane proteins containing extracellular ligand-binding domains and intracellular catalytic domains, and are activated following binding of their cognate ligands. Much of the specificity in signal transduction stems from the specific interaction of RTKs with their ligands, with both receptors and ligands having defined patterns of expression (1). Many of the processes involved in tumor growth, progression and metastasis are mediated by signaling molecules acting downstream of activated RTKs (2).

In particular, several members of the split kinase domain superfamily of RTKs are expressed on solid tumor cells and participate in autocrine loops implicated in cancer growth and survival [e.g. VEGF receptors in melanoma, PDGF receptors in gliomas, KIT in small cell lung cancer and FLT3 in acute myelogenous leukemia] (3–6). Some rare solid tumors are driven by constitutively activated versions of these targets or their ligands (e.g. KIT juxtamembrane mutation in gastrointestinal stromal tumors and collagen IA1/PDGF-B fusions in dermatofibrosarcoma protuberans) (4, 5). Mutations of these split kinase domain RTKs have been particularly evident in hematopoietic malignancies, such as activating mutations of FLT3 in acute myelogenous leukemia (6) and activating translocations of PDGFRβ in chronic myelogenous monocytic leukemia patients (7). Even where mutations have not been detected, inhibition of these split kinase domain RTK targets

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[2] The abbreviations used are: RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; HUVEC, human umbilical vein endothelial cell; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; MTD, maximum tolerated dose; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; MVD, microvessel density; MS, mass spectrometry; PK, pharmacokinetic; PD, pharmacodynamic.

would be anticipated to result in direct effects against cancer cells dependent upon them for growth and survival.

In addition to their direct role in tumor cell growth and survival, several split kinase domain RTKs, namely the VEGF receptors and PDGFRβ, play prominent roles in tumor neoangiogenesis (3). VEGF produced by the tumor cells and associated stromal cells acts on endothelial cells, directly promoting their proliferation, migration, invasion and survival, all critical facets of angiogenesis (8). PDGFRβ is expressed on pericytes, smooth muscle cells which provide mechanical support to vasculature and is also expressed on tumor neovasculature (9, 10). In addition, PDGFRβ is also expressed on fibroblasts in the tumor stromal compartment, which are an important source of VEGF and other growth factors (11). Recent data suggest that combined pharmacological disruption of Flk-1/KDR and PDGFRβ signaling results in profound anti-angiogenic effects (12).

Thus, the signaling cascades generated by the split kinase domain RTKs described above (the VEGF receptors, Flk-1/KDR and Flt-1; the PDGF receptors, PDGFRα and PDGFRβ; KIT and FLT3) directly and indirectly regulate tumor growth, survival and angiogenesis. Inhibiting these targets in concert might be expected to result in broad antitumor efficacy.

To date, signaling inhibitors have generally proven to be relatively well tolerated at efficacious doses in preclinical studies (17). Hence, the emergence of such targeted inhibitors poses a challenge to the traditional cytotoxic paradigm of cancer therapy, whereby agents are administered to the MTD (17). Moreover, rapid tumor debulking in patients (such as that often seen with cytotoxic drugs) may not necessarily be anticipated or observed with this class of agents, making their biological activity in patients more difficult to monitor than for traditional therapies.

The challenge of identifying biologically active doses for molecularly targeted anticancer agents has enhanced the effort to identify biomarkers that can be used to monitor target inhibition or evidence of a surrogate biological effect to guide dose selection. However, until such biomarkers can be identified and validated, an alternative approach to guide clinical development of targeted inhibitors would be to select doses based on a thorough understanding of the pharmacokinetic parameters driving suppression of target activity and consequent tumor responses, initially in preclinical species and, ultimately, in patients.

On the basis of the fact that pharmacokinetic properties such as drug clearance and metabolism often vary substantially between rodents and humans, it is necessary to determine in preclinical efficacy models the plasma levels necessary to inhibit a given target and the duration of inhibition necessary to achieve a biological effect to anticipate exposures necessary to achieve effects in patients. It is anticipated that the information developed from preclinical models will provide guidance in the clinical setting even if there are substantial differences in the shapes of the plasma inhibitor concentration *versus* time profiles in preclinical species and humans.

We report here the results of such preclinical studies for SU11248 (Fig. 1), a novel, selective multitargeted RTK inhibitor that exhibits direct antitumor activity against tumor cells dependent on signaling through PDGFR, KIT, FLT3, or VEGFR for proliferation/survival in addition to antiangiogenic activity through its potent inhibition of VEGFR and PDGFR signaling.

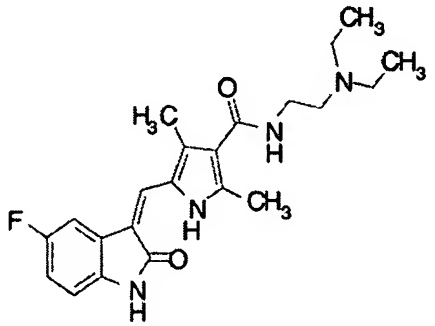

Fig. 1 Chemical structure of SU11248.

Using biochemical and biological readouts, we evaluated the PK/PD relationship for SU11248-induced inhibition of its target RTKs in rodent xenograft models. These data were subsequently integrated with tumor efficacy data to elucidate the relationship between plasma inhibitor concentration necessary to effect target modulation and duration of target inhibition necessary to effect a tumor response to SU11248 treatment. The conclusions from these studies are currently being used to help guide early clinical evaluation of this compound.

MATERIALS AND METHODS

Compounds. SU11248 was synthesized at SUGEN, Inc. ZD1839 is a selective small molecule inhibitor of EGFR tyrosine kinase activity of which the structure and activity have been reported previously (18).

Cells. Unless otherwise indicated, cell culture reagents were obtained from Life Technologies, Inc. (Gaithersburg, MD). A431 (human epidermoid carcinoma), A375 (human melanoma), Colo205 (human colon carcinoma), H460 (human non-small cell lung carcinoma), SF763T and SF767T (human glioma), and C6 (rat glioma) cells were obtained and cultured as described previously (12, 19). MDA-MB-435 (human breast carcinoma) and HT-29 (human colorectal carcinoma) cells were obtained from and cultured as recommended by the American Type Culture Collection (Bethesda, MD). NIH-3T3 mouse fibroblast cells engineered to overexpress Flk-1, human PDGFRα or β, or human EGFR (12) were cultured in DMEM supplemented with 10% FBS and 2 mM glutamine. HUVECs were obtained from Clonetics (San Diego, CA) and cultured as recommended by the supplier. PC-3M2AC6 cells, derived from the human prostate cancer cell line PC-3M and engineered to express luciferase, were obtained from Xenogen Corporation (Alameda, CA) and maintained in RPMI 1640 containing 10% FBS.

***In Vitro* Activity Assays.** Biochemical assays to determine the activity of SU11248 against different protein kinases were performed as described previously (12). Ki values for SU11248 against Flk-1, PDGFRβ, and FGFR1 were determined as described previously (12) using glutathione *S*-transferase-fusion proteins containing the complete cytoplasmic domain of the RTK. Cellular assays to directly determine the ability of SU11248 to inhibit ligand-dependent RTK phosphorylation or cell proliferation and mitogenic responses were performed as described previously (12, 20, 21) using serum-starved cells

stimulated with 40 ng/ml $VEGF_{165}$ (Flk-1/KDR), 0.5 μg/ml basic FGF (FGFR), or 50 ng/ml PDGF-AA (PDGFRα) or PDGF-BB (PDGFRβ).

Mouse Xenograft Models. Unless otherwise indicated, female *nu/nu* mice (8–12 weeks old, 25 grams), obtained from Charles River (Wilmington, MA), were used for these studies. Animals were maintained under clean-room conditions in sterile filter-top cages with Aspen Chip bedding housed on HEPA-filtered ventilated racks. Animals received sterile rodent chow and water *ad libitum*. Animal experiments were conducted as described previously (10, 12) in accordance with Institutional Animal Care and Use Committee guidelines in the SUGEN Animal Facility, which has been accredited by Association for Assessment and Accreditation of Laboratory Animal Care International.

Briefly, 3–5 × 10^6 tumor cells were implanted s.c. into the hind flank region of mice on day 0 as described previously (12). Daily treatment of tumor-bearing mice with oral administration of SU11248 as a carboxymethyl cellulose suspension or as a citrate buffered (pH 3.5) solution was initiated once the tumors reached the indicated average size. Tumor growth was evaluated based on twice-weekly measurement of tumor volume as described previously (10, 12). Typically, studies were terminated when tumors in vehicle-treated animals reached an average size of 1000 mm^3 or when the tumors were judged to adversely effect the well being of the animals. Because tumors were established and often large at the time treatment was initiated, percentage of inhibition of tumor growth after initiation of treatment with SU11248 was calculated as $100 \times \{1 - [(\text{tumor volume}_{\text{final}} - \text{tumor volume}_{\text{initial}}$ for the SU11248-treated group)/(tumor $\text{volume}_{\text{final}} - \text{tumor volume}_{\text{initial}}$ for the vehicle-treated group)]}.

MVD. Tumor specimens to be evaluated for MVD were harvested and fixed in 10% buffered formalin for 24 h before being transferred to 70% ethanol. The specimens were subsequently paraffin-embedded and sectioned. General tissue morphology and mitotic figures were visualized by H&E staining. Tumor microvessels were visualized using immunohistochemical detection of CD31 using a 1:100 dilution of the rat anti-mouse monoclonal antibody MEC 13.3 (BD PharMingen, San Diego, CA) and visualized using the labeled streptavidin biotin plus KIT (DAKO, Carpinteria, CA). All of the immunostained sections were counterstained using hematoxylin. Three or four fields per tumor were scored (in blinded fashion) for tumor microvessel elements at ×400 magnification by two blinded observers, and tumor microvessel densities (*i.e.*, number of microvessel elements/field) were calculated.

Xenogen IVIS Imaging System. PC-3M cells expressing luciferase were resuspended in PBS (5 × 10^6 cells/100 μl), combined with an equal volume of Basement Matrigel Matrix (Becton Dickinson, Bedford, MA), and injected s.c. into the hind flank region of male *nu/nu* mice. Tumors were allowed to grow untreated until they reached 200–300 mm^3, at which point the mice underwent initial imaging. Before imaging, mice were given a 150 mg/kg dose of D-luciferin (Xenogen) by i.p. injection immediately before being anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Mice were imaged 15 min after luciferin administration using the IVIS system to determine total flux (photons/sec) of emitted light as a measure of the relative number of viable tumor cells in the tumor. Data were analyzed using Xenogen LivingImageR software.

***In Vivo* Target Modulation Studies.** *In vivo* target modulation studies to determine the effect of inhibitor treatment on the phosphorylation of target RTKs expressed on tumor cells were performed as described previously (13, 22). Briefly, implanted tumor cells expressing the desired target (A375 melanoma cells for KDR, SF767T glioma cells for PDGFR, and A431 epidermoid cells for EGFR) were allowed to grow untreated to a size of 300–500 mm^3. Mice were then administered a single oral dose of SU11248 at the indicated concentration. At the indicated times after dosing, individual mice were sacrificed, their tumors resected, and a blood sample taken by cardiac puncture using a syringe primed with heparin sulfate.

Resected tumors were snap frozen on dry ice, pulverized using a liquid nitrogen-cooled cryomortar and pestle, and lysed in cold HTNG buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, 10% v/v glycerol, 1% Triton X-100, 1% sodium orthovanadate, 2 mM NaF, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 2 μg/ml pepstatin A]. Total PDGFR, EGFR, FGFR, or phosphotyrosine-containing protein (for Flk-1/KDR) was immunoprecipitated from individual powdered tumors, and the amount of phosphorylated and total RTK in each sample determined by Western blot analysis. The extent of phosphorylation in tumors resected from SU11248-treated animals was typically compared with that in tumors resected from untreated or vehicle-treated animals, either predose or at the same time point, based on visual inspection of films after Western blot analysis. In this evaluation, compound was judged to have had a weak (<25% inhibition), moderate (25–75% inhibition), or strong (>75% inhibition) effect on receptor phosphorylation based on comparison of the estimated ratio of phosphorylated to total receptor in mice receiving different treatments. In experiments to evaluate EGFR phosphorylation, epidermal growth factor (50 μg) was injected into the tail vein of each mouse 30 min before sacrifice.

Plasma samples, obtained by centrifuging individual blood samples at 3000 × *g* for 10 min at 4°C in an Eppendorf 5417R refrigerated centrifuge, were stored at −80°C until they could be analyzed for drug concentration using liquid chromatography with tandem mass spectrometric detection. Briefly, plasma samples (100 μl) or SU11248 standards in mouse plasma were mixed with acetonitrile (400 μl), the mixture filtered using a 3M Empore 96-Well PPT Filter Plate (3M, Minneapolis, MN), and each filtrate sample was injected onto a LC/MS/MS system where separation occurred on a BDS Hypersil C-18 (5 μm, 50 × 4.6 mm) reverse-phase high-performance liquid chromatography column (Keystone Scientific, Foster City, CA). The amount of inhibitor and the internal standard (DL-propranolol hydrochloride) in each mouse plasma sample was quantified based on standard curves generated using known amounts of compound. In these studies, plasma inhibitor concentration is calculated as the combined concentration of SU11248 and its primary metabolite, which has biological activity comparable with SU11248 *in vitro* and *in vivo*.[3]

Miles Assay for Vascular Permeability. The Miles assay for vascular permeability (23) was adapted to athymic mice as

[3] D. Mendel, D. Laird, K. G. Moss, J. O. Haznedar, and J. Sukbuntherneg, unpublished data.

Table 1 Summary of the *in vitro* activity of SU11248

Receptor	Biochemical K_i (μM)[a]	Cellular IC_{50}: Receptor phosphorylation (μM)[b]	Cellular IC_{50}: Ligand-dependent proliferation (μM)[c]
Flk-1/KDR	0.009 ± 0.002	0.01	0.004 ± 0.002
PDGFRβ	0.008 ± 0.003	0.01	0.039 ± 0.013
PDGFRα	ND[d]	ND	0.069 ± 0.015
FGFR1	0.83 ± 0.12	ND	0.88 ± 0.15

[a] Determined using recombinant enzyme.
[b] Determined after ligand stimulation of serum-starved NIH-3T3 cells engineered to express Flk-1 or PDGFRβ.
[c] Determined using serum-starved HUVECs (KDR and FGFR) or NIH-3T3 cells engineered to express PDGFRα or PDGFRβ
[d] ND, not determined.

follows. Mice were given a single oral dose of SU11248 or vehicle alone. Seven, 11, 15, or 23 h later, 100 μl of a 2.2% (w/v) solution of FITC-Dextran (150 kDa; Sigma) was administered i.v. via the tail vein. One h later, mice were injected intradermally with 400 ng of VEGF (R&D Systems, Minneapolis, MN) dissolved in 20 μl of PBS or PBS alone (duplicate points for a total of four injection sites per mouse). Thirty min later, VEGF-dependent dye leakage from the vasculature into skin was imaged using a fluorescent stereomicroscope (Leica MZ7.5; Leica Microsystems Inc., Bannockburn, IL) and quantitatively scored using a Photoshop plug-in (Image Processing Tool kit 4.0; Reindeer Games Inc., Raleigh, NC). For each animal a vascular permeability score was calculated [VEGF-dependent fluorescence divided by control (PBS) fluorescence]. Scores for individual animals in a treatment group were averaged to derive a group vascular permeability score. Percentage of inhibition relative to vehicle-treated groups was then calculated.

RESULTS

In Vitro Studies

The *in vitro* activity of SU11248 was evaluated using biochemical assays, and ligand-dependent cell kinase and cell proliferation assays (Table 1). In biochemical assays, SU11248 exhibited competitive inhibition (with regard to ATP) against Flk-1 and PDGFRβ with K_i values of 0.009 μM and 0.008 μM, respectively. SU11248 was also a competitive, albeit less potent, inhibitor of FGFR-1 tyrosine kinase activity, with a K_i value of 0.83 μM. In addition to these three structurally related split kinase domain RTKs, the activity of SU11248 has also been evaluated against a broad panel of additional tyrosine and serine/threonine kinases. In these biochemical assays, the IC_{50} values for SU11248 were generally at least 10-fold higher than those for Flk-1 and PDGFR (*e.g.*, IC_{50} values of: >10 μM for EGFR and Cdk2; 4 μM for Met; 2.4 μM for IGFR-1; 0.8 μM for Abl; and 0.6 μM for src). However, it should be noted that whereas SU11248 does not inhibit RTKs outside the split kinase domain RTK subfamily, it is a potent inhibitor of KIT[4] and FLT3 (22), two other split kinase domain RTKs.

[4] T. J. Abrams, L. B. Lee, L. J. Murray, N. K. Pryer, and J. M. Cherrington, manuscript in prep.

The ability of SU11248 to inhibit ligand-dependent RTK phosphorylation in cells was evaluated using cell lines that normally express or were engineered to express high levels of the RTK of interest. As shown in Table 1, SU11248 inhibited VEGF-dependent Flk-1 tyrosine phosphorylation and PDGF-dependent PDGFRβ phosphorylation with IC_{50} values of ~0.01 μM for both RTKs. These values are similar to the K_i values determined for the two RTKs in the biochemical assays and confirm that SU11248 has equal activity against the two targets.

In addition to evaluating the ability of SU11248 to inhibit ligand-dependent receptor phosphorylation in cells, the effect of SU11248 on ligand-dependent proliferation of cells was examined. As shown in Table 1, SU11248 inhibited VEGF- and FGF-induced proliferation of HUVECs with IC_{50} values of 0.04 and 0.7 μM, respectively. SU11248 also inhibited PDGF-induced proliferation of NIH-3T3 cells overexpressing PDGFRβ or PDGFRα with IC_{50} values of 0.03 and 0.07 μM, respectively. Thus, the ability of SU11248 to inhibit cell mitogenic and proliferative responses to ligand stimulation was consistent with its relative activity against the specific RTK, indicating that the cellular activity of SU11248 mirrors its biochemical activity.

In Vivo Studies

Efficacy Studies in Mouse Xenograft Models. The *in vivo* efficacy of SU11248 was studied in s.c. xenograft models using human (HT-29, A431, Colo205, H-460, SF763T, A375, and MDA-MB-435) and rat (C6) tumor cells derived from various different tumor types. After implantation into the hind flank region of athymic mice, tumors were allowed to grow to a size of 100–550 mm^3 before starting daily oral treatment with SU11248.

The results of representative studies with once daily oral dosing for each of the tumor models tested are summarized in Table 2, and examples are shown in Fig. 2. These data indicate that SU11248 was efficacious against all of the tumor types tested, even when treatment was not initiated until the tumors were quite large. In three of the models (A431, Colo205, and C6), the dose response for SU11248 was also evaluated. In each of these tumor models SU11248 had a dose-dependent effect, with the 80 mg/kg/day dose providing little, if any, additional benefit compared with 40 mg/kg/day, but the 40 mg/kg/day dose being more effective than 20 mg/kg/day. Thus, for all of the models tested, 40 mg/kg/day was considered to be a very efficacious dose.

To determine whether SU11248 treatment could eradicate tumors, mice bearing established A431 tumors (100 mm^3) were dosed with SU11248 at 80 mg/kg/day for 21 days. On the basis of caliper measurements, complete tumor regression occurred in six of the eight mice, and the tumors did not regrow during a 110-day observation period after the end of treatment. In the two animals in which the tumors did not fully regress, the tumors were allowed to regrow to a large size (2000–3000 mm^3) before reinitiating treatment with SU11248. Both tumors regressed in response to this second round of treatment (data not shown), indicating that SU11248 remained efficacious against tumors that were not fully regressed during the first round of treatment.

In addition to measuring changes in tumor volume, the effect of SU11248 treatment in representative experiments was evaluated based on histological and immunohistochemical analysis of tumors resected from treated and untreated animals. One observation from these studies was that SU11248 treatment

Table 2 SU11248 treatment effectively inhibits the growth of established tumor xenografts

Tumors were established as s.c. xenografts. Number of cells implanted per animal: SF763T and C6, 3×10^6; MDA-MB-435, 1×10^7; all other tumors. 5×10^6. Once daily treatment with oral SU11248 was initiated at the indicated dosages, when tumors had reached the indicted sizes. Percentage of growth inhibition values relative to vehicle-treated controls are indicated for cases in which overall effect was growth inhibition; maximum regression relative to tumor size at which treatment was initiated are indicated for cases in which overall effect was regression.

Cell line	Tumor type	Initial tumor volume (mm^3)	Dose (mg/kg/day)	% Growth inhibition	% Regression
HT-29	Colon	360	40		62 (d74)
A431	Epidermoid	400	80		30 (d40)
			40	93 (d36)	
			20	65 (d36)	
Colo205	Colon	250	80		38 (d35)
			40		13 (d35)
			20	55 (d35)	
H460	NSCLC	300	80	84 (d25)	
SF763T	Glioma	550	80	79 (d30)	
C6	Rat glioma	330	80	88 (d25)	
			40	82 (d25)	
		110	40	72 (d25)	
			20	41 (d25)	
A375	Melanoma	230	40	64 (d74)	
MDA-MB-435	Breast	150	80	71 (d73)	
			20	11 (d73)	

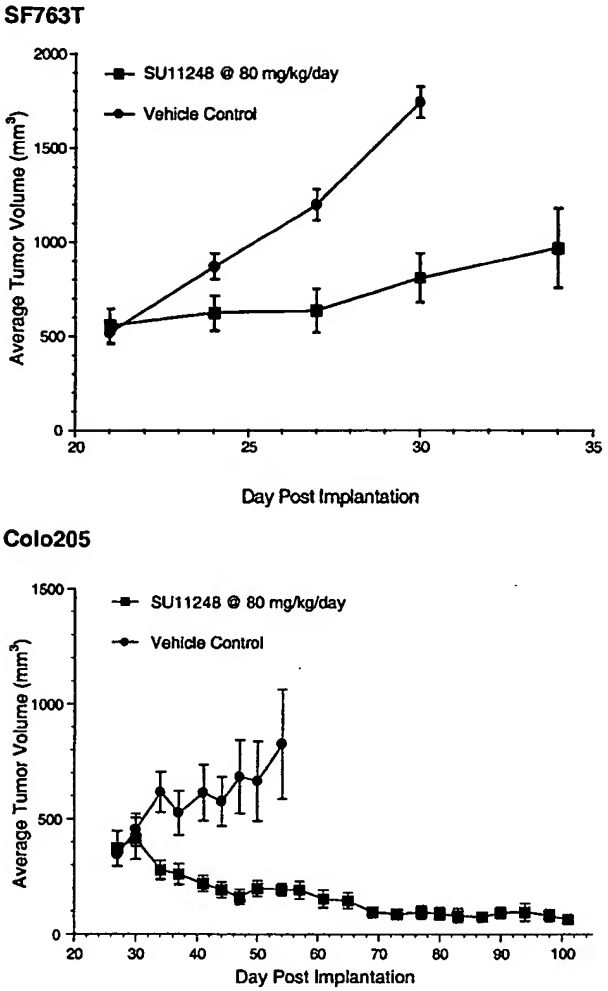

Fig. 2 SU11248 inhibits the growth of established SF763T and Colo205 tumor xenografts in athymic mice. SF763T human glioma (*A*) or Colo205 human colon (*B*) tumors were established in athymic mice. Daily oral administration of SU11248 at 80 mg/kg/day was initiated when the tumors reached an average size of 550 mm^3 (SF763T) or 350 mm^3 (Colo205) and continued through the end of the experiment. Tumor volume was measured on the indicated days, with the mean tumor volume indicated for groups of 8 (treated) or 16 (vehicle control) animals; *bars*, ±SE.

resulted in a decrease in tumor MVD, which is taken to be an indication of antiangiogenic activity consistent with the demonstration that SU11248 inhibits VEGF-dependent mitogenic response of HUVECs *in vitro* (Table 1). As an example, SU11248 treatment resulted in a ~40% reduction in MVD (24.2 ± 4.1 *versus* 39.3 ± 5.7; $P < 0.05$) in SF763T glioma tumors (Fig. 3), which, like C6 tumors, exhibited a greatly slowed growth rate but did not undergo regression or stasis after treatment with SU11248. Thus, these data provide direct evidence of the antiangiogenic activity of SU11248, even in a tumor model that does not regress in response to SU11248 treatment.

Another observation from the histological studies was that in tumors derived from some cell lines (*e.g.*, H-460 lung carcinoma cells) SU11248 treatment resulted in extensive histological destruction of the tumor mass (data not shown) although there was no apparent reduction in tumor size (Table 2; 84% growth inhibition but no evidence of regression). Thus, it was possible to have extensive antitumor activity although there was little or no evidence of tumor regression, a phenomenon we have noted previously with another inhibitor targeting VEGFR and PDGFR (13). Because of the potential to underestimate the overall effect (growth inhibition, stasis, or regression) based on external measurements of tumor volume, we have investigated alternative methods of evaluating antitumor activity in mouse xenograft models. In particular, we have investigated the use of a noninvasive imaging technology, the Xenogen *in vivo* imaging system (25), to monitor antitumor effects in individual mice throughout the course of a study. This technology relies on measurement of luminescence produced by live tumor cells engineered to express luciferase as a way to evaluate viability of tumor cells.

The results of a representative experiment in which the efficacy of SU11248 was evaluated in male *nu/nu* mice bearing luciferase-expressing PC-3M (human prostate) tumors are shown in Fig. 4. In this experiment, caliper measurements of the tumors suggested that SU11248 treatment resulted in a complete inhibition of additional tumor growth (*i.e.*, stasis) but no reduction in tumor size (Fig. 4*A*). In contrast, detection of photon emissions (Fig. 4, *B* and *C*) as an indicator of the relative

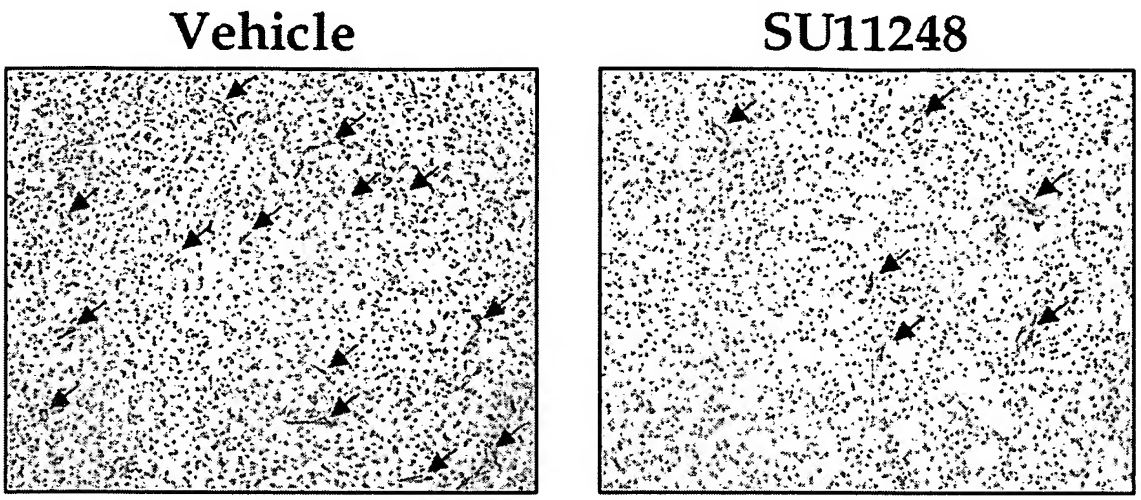

Fig. 3 SU11248 treatment results in inhibition of SF763T tumor MVD. Mice bearing established (500 mm^3) SF763T tumors were treated with SU11248 at 80 mg/kg/day or with vehicle. Tumors were harvested 13 days after initiation of treatment (9 days in the case of vehicle because of rapid tumor growth). Tumors were fixed, paraffin-embedded, sectioned, immunostained for CD31, and counterstained with hematoxylin. Representative ×400 fields are shown. Arrows indicate CD31-positive cells (vessel elements).

number of viable tumor cells revealed that, in comparison with when treatment was initiated (day 14), the SU11248-treated mice had a reduction in viable tumor cells on day 43, the last day of treatment. Thus, the Xenogen system, like histological evaluation, provided a more accurate indication of the antitumor activity of SU11248 than did measurement of tumor volume. It should be noted that in a second experiment, in which dosing with SU11248 was initiated when tumors were smaller (~100 mm^3), reduction in both tumor volume and photon emission was observed, suggesting that the lack of apparent decrease in tumor size in the first study could reflect an inability to efficiently clear debris from large PC-3M tumors.

Inhibition of Flk-1/KDR and PDGFR Phosphorylation *in Vivo*. Most of the tumor cell lines used for the efficacy studies do not express RTKs targeted by SU11248. Therefore, although SU11248 can exert direct antitumor activity against tumors expressing target RTK(s) required for tumor cell proliferation or survival, it is assumed that the antitumor activity in these models is primarily mediated through the antiangiogenic activity of SU11248. Because the VEGF and PDGF receptors are critical mediators of tumor angiogenesis, and are potently modulated by SU11248 *in vitro*, we investigated the PK/PD relationship underlying the activity of SU11248 against these targets *in vivo*. Although the primary antiangiogenic target for SU11248 *in vivo* will be VEGF and PDGF receptors expressed on the endothelium and/or stroma of all solid tumors, we initially investigated the effect of SU11248 on phosphorylation of target RTKs expressed on tumor cells themselves. This was done to ensure that enough receptor was available to evaluate its phosphorylation status after immunoprecipitation from tumor lysates and Western blot analysis.

The ability of a single oral dose of SU11248 to inhibit Flk-1/KDR and PDGFR phosphorylation *in vivo* was evaluated in athymic mice bearing A375 melanoma tumors, which express KDR, or SF767T tumors, which express PDGFRβ. As shown in Fig. 5*A*, oral administration of SU11248 at 40 mg/kg caused substantial inhibition of VEGF and PDGF receptor phosphorylation when compared with the amount of receptor phosphorylation in tumors taken from untreated or vehicle-treated mice. From the data shown in the lower panel of Fig. 5*A*, it is also possible to determine that inhibition of Flk-1/KDR phosphorylation extends for 12 h, but not 24 h, after administration of a 40 mg/kg dose of SU11248. In contrast, there is a much less dramatic and shorter lived effect after administration of a 5 mg/kg dose of SU11248 (Fig. 5*A*). With this dose, inhibition of Flk-1/KDR phosphorylation is only evident 1.5 h after dosing, which corresponds to the time at which inhibitor plasma concentration is maximal (Fig. 6). On the basis of the results of more extensive studies similar to those shown in Fig. 5*A*, it has been possible to demonstrate dose- and time-dependent inhibition of KDR and PDGFRβ phosphorylation *in vivo*, with the degree of inhibition and the duration of inhibition increasing with increasing dose. In separate experiments, we were also able to demonstrate that SU11248 inhibited downstream signaling events (*e.g.*, phosphorylation of peritoneal lymphocyte γ and phosphatidylinositol 3′-kinase, immediate downstream indicators of PDGFR activation) in tumors removed from SU11248-treated animals (data not shown). Thus, these data provided direct evidence that oral administration of SU11248 inhibited target receptor phosphorylation and signaling *in vivo*.

As discussed above, PDGFRβ is expressed on pericytes, stromal cells, and fibroblasts that would support neoangiogenic vessels in solid tumors. To determine whether inhibition of PDGFRβ phosphorylation could be detected in tumors that did not express the RTK on the tumor cells, but rather only on the host pericytes, stromal cells, and fibroblasts, the ability of SU11248 to inhibit PDGFR phosphorylation was also evaluated in tumors derived from cells that do not express the receptor. Colo205 cells, which do not express PDGFRβ based on Western blot analysis (data not shown), were used for these experiments.

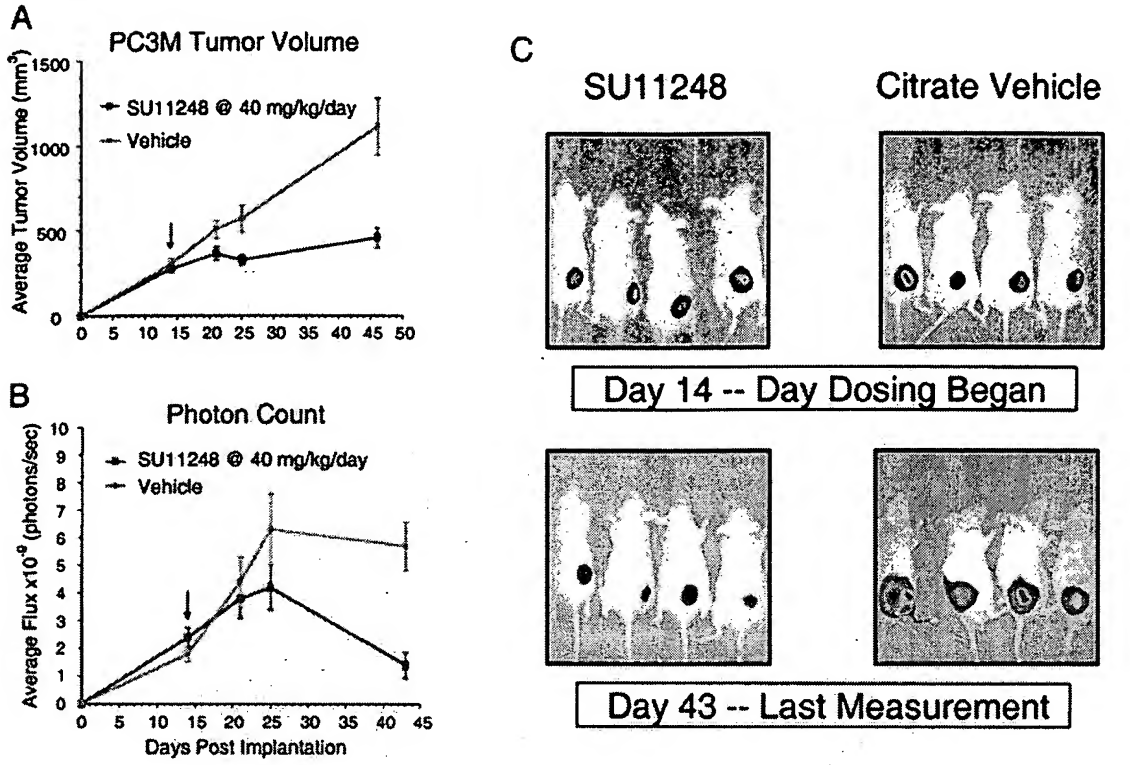

Fig. 4 Evaluation of SU11248 effects on the growth of established PC-3M tumors based on physical measurement or photon emission. PC-3M tumor cells expressing luciferase (5×10^6 cells/mouse) were implanted s.c. into the hind flank region of male athymic mice. Daily oral administration of SU11248 at 40 mg/kg/day was initiated when the tumors reached an average size of 300 mm^3. Tumor growth was subsequently monitored based on (*A*) external measurement using caliper, or (*B*) determination of emitted bioluminescence (photons/sec) 15 min after i.p. administration of D-luciferin (150 mg/kg). *C*, photon emission from individual mice was visualized before administration of the first dose of SU11248 or vehicle (day 14) and after the last measurement (day 43). Red color indicates more fluorescence indicative of a greater number of live tumor cells; *bars*, ±SE.

As shown in Fig. 5*B*, oral administration of SU11248 inhibited PDGFRβ phosphorylation in Colo205 tumors. Thus, because Colo205 cells do not express PDGFRβ, these results indicate that oral administration of SU11248 inhibited PDGFRβ phosphorylation on host cells that support developing vessels within the tumor.

Determination of an Active Plasma Concentration of SU11248. To determine the target plasma concentration for SU11248, the minimum plasma concentration of SU11248 required to inhibit receptor phosphorylation *in vivo* was determined. This was done by conducting target modulation experiments similar to those shown in Fig. 5*A*, but using different dose levels of SU11248 and harvesting samples at different times after dosing to thoroughly investigate the dose and time dependence of the inhibition of Flk-1/KDR and PDGFR phosphorylation *in vivo*. In these experiments, plasma samples were collected at the same time tumors were harvested to correlate plasma inhibitor concentration with inhibition of receptor phosphorylation in the harvested tumors.

The results of these studies, which are summarized in Table 3, indicate that inhibition of Flk-1/KDR and PDGFRβ phosphorylation in tumors was dose- and time-dependent. In our initial, dose-range finding studies, we determined that after a single oral administration of SU11248 at 40 mg/kg, a dose that was very efficacious when administered daily, substantial inhibition of receptor phosphorylation was evident as long as 12 h, but not 24 h, after dosing (Fig. 5*A*, *bottom*). It was not until doses reached 80 mg/kg that inhibition of receptor phosphorylation was detected for the full 24 h dosing interval (Fig. 5*C*).

Because the extent of receptor phosphorylation necessary to result in biological (antitumor) activity it is not known, the conclusion that continuous target inhibition was not achieved at a very efficacious dose of SU11248 was additionally tested using a functional assay to directly assess inhibition of VEGF-induced vascular permeability in the skin of athymic mice (13). Thus, this assay provides a functional readout for VEGF receptor activity (rather than phosphorylation) in the vascular bed (rather than tumor cells), which is the target tissue for the antiangiogenic activity of this inhibitor. The results of these assays confirmed that SU11248 did not inhibit Flk-1/KDR activity for the full 24-h dosing period at the 40 mg/kg dose. As summarized in Table 3, essentially complete inhibition of VEGF-induced vascular permeability was observed for 12 h after a 40 mg/kg dose, with some inhibition seen 16 h and no inhibition seen 24 h after the dose. Thus, both the target modulation and vascular permeability data from mice dosed at a very efficacious dose (40 mg/kg) indicate that constant inhibition of

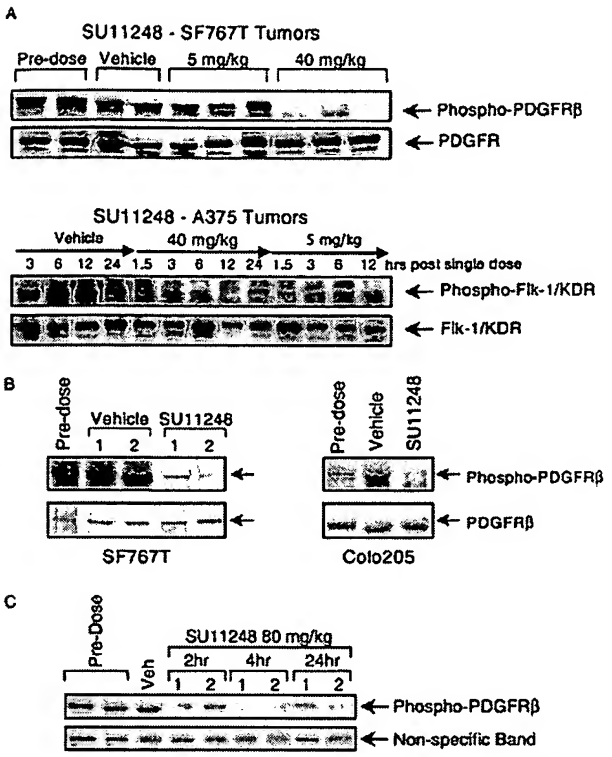

Fig. 5 SU11248 treatment causes dose- and time-dependent inhibition of Flk-1/KDR and PDGFRβ phosphorylation *in vivo*. SF767T, A375, or Colo205 cells (5×10^6 cells/mouse) were implanted s.c. into the hind flank region of athymic mice. Mice bearing established (300–500 mm^3) tumors were treated with a single oral dose of SU11248 at the indicated dose or vehicle alone. Each *lane* represents a separate animal. *A*, mice bearing SF767T tumors were sacrificed 2 h after dosing. PDGFRβ was immunoprecipitated from tumor lysates, and Western blots probed for phosphotyrosine (*Phospho-PDGFRβ*) or total PDGFRβ (*PDGFRβ*). Alternatively, mice bearing A375 tumors were sacrificed at the indicated times after treatment with SU11248. Total phosphotyrosine-containing proteins were immunoprecipitated from tumor lysates and Western blots probed for Flk-1/KDR. Total Flk-1/KDR was determined in a second sample of the same lysate. *B*, PDGFRβ was immunoprecipitated from tumor lysates prepared 4 h after oral administration of an 80 mg/kg dose of SU11248 to mice bearing SF763T tumors, which express PDGFRβ on the tumor cells, or Colo205 tumors, which do not express PDGFRβ. Phospho-PDGFRβ and total PDGFRβ were detected as described above. *C*, SF767T tumors were resected at the indicated time after oral administration of SU11248 at 80 mg/kg. In this experiment phosphotyrosine-containing proteins were immunoprecipitated from tumor lysates, and Western blots probed for PDGFRβ. Each *lane* represents a single animal, and the data shown are representative of at least two experiments.

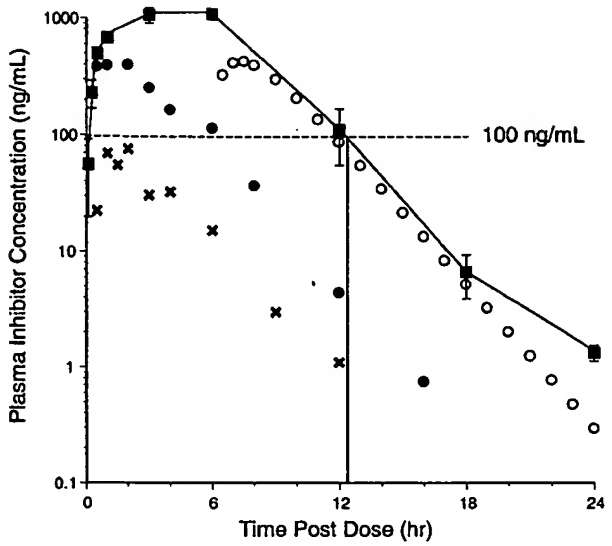

Fig. 6 Plasma inhibitor concentration *versus* time profile in mice given an oral dose of SU11248. Athymic mice were given a single oral dose of SU11248. At the indicated times after dosing, plasma samples were obtained from terminal bleeds of individual mice, and the concentration of inhibitor in each sample determined by LC/MS/MS. (■), data from PK study with mice dosed at 40 mg/kg; (●), data from target modulation studies at 20 mg/kg; (○), simulated projection of expected plasma inhibitor concentrations in mice given a second 20 mg/kg dose 6 h after the first dose; (×), data from target modulation studies at 5 mg/kg. For the PK study, each point represents mean for groups of three animals; *bars*, ±SE. For the target modulation studies, each point represents individual animals.

Flk-1/KDR and PDGFRβ kinase activity is not required for SU11248 to have potent antitumor activity.

The minimum plasma concentration of inhibitor required to block Flk-1/KDR and PDGFRβ phosphorylation *in vivo* was determined using target modulation experiments similar to those described above, but with low doses of SU11248. By doing a series of experiments in which the dose of inhibitor was altered and tumor and plasma samples were collected at different times after dosing, we were able to correlate the extent of inhibition of receptor phosphorylation and plasma concentration of inhibitor for both Flk-1/KDR and PDGFRβ. The results of these experiments indicated that inhibition of both target receptors was obtained at plasma inhibitor concentrations at or above 50–100 ng/ml (data not shown). The similarity of the sensitivity of PDGFRβ and Flk-1/KDR to inhibition by SU11248 *in vivo* is consistent with the biochemical and cellular data. Data from the VEGF-induced vascular permeability assays support the identification of 50–100 ng/ml as the minimum plasma concentration required to inhibit Flk-1/KDR function *in vivo*.

It is worth noting that based on plasma protein binding measurements for SU11248, the free concentration (~5% of total) of inhibitor in the blood at the 50–100 ng/ml target plasma concentration would be in the single digit nanomolar range, which is similar to the concentration required to inhibit ligand-dependent receptor phosphorylation and cellular activity in cell-based assays performed under low serum conditions (Table 1). Thus, there is a good correlation between the *in vitro* and *in vivo* data for SU11248. In addition, based on the plasma concentration *versus* time curves shown in Fig. 6, the plasma concentration of inhibitor stays at or above the target plasma concentration for ~12 h after administration of a very efficacious dose (40 mg/kg), but only for ~6 h at a less efficacious dose of 20 mg/kg, and only approaches the target concentration at C_{max} values at a nonefficacious dose of 5 mg/kg. Thus, there is agreement among the time above the target plasma concentration, the duration of inhibition of target receptor phosphorylation, and the degree of efficacy in mouse xenograft models.

Whereas the above data are highly suggestive that

Table 3 SU11248 exhibits dose- and time-dependent inhibition of target RTK phosphorylation and VEGF-induced vascular permeability *in vivo*

Target modulation studies were performed as described in "Materials and Methods" using mice bearing established A375 (Flk-1/KDR) or SF767T (PDGFRβ) tumors. Data for Flk-1/KDR and PDGFRβ from multiple experiments were combined, and the presence of >50% inhibition relative to vehicle-control animals (based on visual inspection of gels) is indicated. Miles assays to determine inhibition of VEGF-induced vascular permeability were performed as described in "Materials and Methods." The extent of VEGF-dependent dye leakage from the vasculature into skin was quantified, and the data from three individual mice were combined. The percentage of inhibition relative to vehicle-treated control animals is indicated.

	Duration of inhibition							
	Target modulation				Vascular permeability (% Inhibition)			
Dose (mg/kg)	8 h	12 h	16 h	24 h	8 h	12 h	16 h	24 h
80	Yes	Yes	ND[a]	Yes	95	85	94	98
40	Yes	Yes	ND	No	97	96	0	0
20	Yes	Slight	ND	No	93	0	0	4
5	No[b]	No	ND	No	ND	ND	ND	ND

[a] ND, not determined.
[b] Target modulation activity only detected at 4-h timepoint.

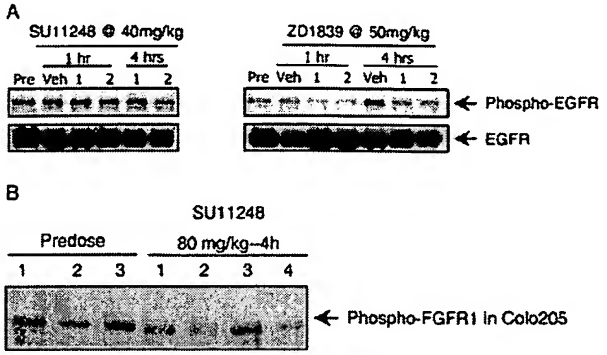

Fig. 7 The selectivity of SU11248 is maintained *in vivo*. *A*, A431 cells (5×10^6 cells/mouse) were implanted s.c. into the hind flank region of athymic mice. Mice bearing established (300–500 mm^3) tumors were given a single oral dose of SU11248 (40 mg/kg), ZD1839 (Iressa; 50 mg/kg), or vehicle alone. Mice were sacrificed at the indicated time after dosing, and each mouse received an i.v. dose of EGF (50 μg) 30 min before sacrifice. EGFR was immunoprecipitated from tumor lysates and Western blots probed for phosphotyrosine (*Phospho-EGFR*) or total EGFR (*EGFR*). *B*. Colo205 cells (5×10^6 cells/mouse) were implanted s.c. into the hind flank region of athymic mice. Mice bearing established (300–500 mm^3) tumors were given a single oral dose of SU11248 (80 mg/kg) or vehicle alone. FGFR-1 was immunoprecipitated from tumors resected 4 h after dosing and Western blot probed for phosphotyrosine to detect phospho-FGFR1. Each *lane* represents a single animal, and the data shown are representative of two experiments.

SU11248 efficacy is driven by maintaining an effective plasma concentration for half of the daily dosing interval, it was important to address the potential therapeutic contributions of C_{Max}-driven antitumor effects. To specifically address this question, dose regimen experiments were carried out in mice bearing established Colo205 tumors, which regress in response to oral administration of SU11248 at a daily dose of 40 mg/kg/day (Table 1). In these experiments, groups of tumor-bearing mice were given higher doses of SU11248 (up to 160 mg/kg/dose), to achieve high maximum plasma concentrations, administered infrequently (once or twice weekly to reduce the relative time plasma inhibitor concentrations were maintained above the 50–100 ng/ml target level). Alternatively, mice were given lower doses of SU11248 (down to 10 mg/kg/dose) administered twice daily to maintain time above the target plasma concentration but reduce maximum plasma levels. The results of these studies indicate that once or twice weekly administration of even the highest dose (160 mg/kg/dose) did not regress the tumors, and in fact did not statistically significantly inhibit tumor growth. In contrast, twice daily dosing of SU11248 at 20 mg/kg/dose (a dose that delayed tumor growth when administered once daily) resulted in plasma inhibitor levels at or above the target plasma concentration for ~12 h (Fig. 6) and induced tumor regression comparable with that seen with doses of 40 or 80 mg/kg administered once daily. Thus, the dose regimen studies support the conclusion that maintenance of the target plasma concentration is responsible for driving efficacy of this compound. Taken together, the target modulation and dose regimen data indicate that an efficacious exposure would be achieved if plasma concentrations of inhibitor are maintained at or above the 100 ng/ml level for 12 h on a daily dosing regimen.

***In Vivo* Selectivity of SU11248.** In mice, SU11248 achieves a high maximum plasma concentration after oral administration, and it is rapidly eliminated (Fig. 6). Consequently, high plasma inhibitor levels are achieved for relatively short periods of time at doses necessary to maintain target plasma concentrations (50–100 ng/ml) for 12 h. To demonstrate that SU11248 maintains its *in vitro* selectivity *in vivo*, target modulation studies similar to those described above were conducted to evaluate its activity against EGFR and FGFR-1 in tumors. These targets were selected because they represent different levels of sensitivity to the compound with SU11248 inhibiting EGFR with an IC_{50} > 10 μM and FGFR-1 with an IC_{50} = 0.8 μM in biochemical assays (Table 1).

As shown in Fig. 7*A*, an efficacious dose of SU11248 (40 mg/kg) had no effect on EGFR phosphorylation *in vivo* although tumors were resected 2 h after dosing, at a time when plasma inhibitor levels are maximal (see Fig. 6). In contrast, ZD1839 (Iressa), a potent and selective EGFR inhibitor, inhibited EGFR phosphorylation. In similar experiments (Fig. 7*B*), SU11248 was able to inhibit FGFR1 phosphorylation. However, in the experiment shown in Fig. 7*B*, as well as in additional studies (data not shown), inhibition of FGFR-1 was variable and only seen at plasma levels of ~800 ng/ml, which is much higher than the ~100 ng/ml required to inhibit Flk-1/KDR and PDGFRβ (see above).

DISCUSSION

There is great hope that molecularly targeted therapies such as RTK inhibitors will offer a new and substantially different approach to the treatment of human cancers. This approach focuses on using agents to selectively target susceptible aspects of tumor biology whereas having relatively little effect on normal adult physiological functions. However, a key component of this scenario depends on the ability to identify clini-

cally relevant doses that provide maximum efficacy, rather than relying on identification of a MTD to guide dosing as has been done for conventional cytotoxic agents.

Whereas the potential of such a strategy is well recognized, it has been challenging to reduce this to practice. Even in the case of Gleevec (STI-571), a c-Abl inhibitor that has demonstrated potent activity in chronic myelogenous leukemia patients, the Phase II clinical dose was derived from a typical Phase I study designed to identify the MTD (26). However, because Gleevec caused such rapid and easily detected responses in patients (*i.e.,* decrease in blast counts), it was possible to identify a biologically active dose to take forward into registrational studies (26). Retrospectively, it has been possible to demonstrate that plasma levels of the inhibitor achieved at efficacious doses in patients were similar to those achieved at efficacious doses in preclinical models (27).

Similarly, the Phase II dose for Iressa (ZD1839), a potent EGFR inhibitor, has been selected based on determination of an MTD. However, it should be noted that elegant functional studies to identify a biomarker based on inhibition of mitogen-activated protein kinase activity in skin punch biopsies were conducted during the Phase I trials with this compound (28). Consequently, functional data have confirmed that the dose selected from the Phase I study did inhibit the target, or at least downstream signaling events (mitogen-activated protein kinase activation) influenced by inhibiting the target (EGFR).

The primary goal of the studies described in this report was to prospectively identify in preclinical models the PK/PD factors that contribute to the efficacy of a selective RTK inhibitor, SU11248, targeting the VEGF and PDGF receptors. This was accomplished by thoroughly evaluating the extent and duration of target receptor inhibition *in vivo* at efficacious, subefficacious, and nonefficacious doses of SU11248. The ultimate goal of these studies was to determine pharmacokinetic exposures required to drive efficacy in the clinical setting to guide dose selection during Phase I studies.

As indicated above, SU11248 selectively inhibits signaling through the split kinase domain VEGF, PDGF, KIT and FLT3 receptors. Thus, SU11248 is able to exert direct antitumor activity against tumor cells that rely on these RTKs for proliferation and/or survival, but is also able to indirectly inhibit tumor growth by inhibiting angiogenesis. Most of the tumor cell lines used for the efficacy studies reported here do not express RTKs targeted by SU11248. Thus, it is likely that the PK/PD relationship described here is related to the anti-angiogenic activity of SU11248 resulting from inhibition of the VEGF and PDGF receptors.

Thus, the most important finding from these studies was the determination that in athymic mice 50–100 ng/ml is the minimum plasma concentration of inhibitor required to inhibit phosphorylation of VEGF and PDGF receptors, the primary targets responsible for the antiangiogenic activity of SU11248. Because the plasma protein binding of SU11248 is comparable in plasma from athymic mice and humans, we would expect to inhibit these targets in patients administered SU11248 if we achieve a plasma inhibitor concentration of 50–100 ng/ml. Results from these and other studies also indicate that SU11248 retains its selectivity *versus* other RTKs at these plasma levels, as indicated by the observation that FGFR and EGFR phosphorylation were not substantially inhibited in athymic mice even at plasma levels ~10-fold higher than the 100 ng/ml target.

The other primary finding from this study was that it is not necessary to maintain continuous inhibition of the target receptors to achieve efficacy in the murine models. At an efficacious dose (*e.g.,* 40 mg/kg/day), VEGFR and PDGFR phosphorylation was substantially inhibited for at least 12 h, but recovered by the 24 h time point, before the next dosing. This result confirms and extends our previous findings with SU6668, a different RTK inhibitor that targets the VEGF, PDGF, and FGF receptors (13). Thus, based on the observation that both VEGF and PDGF receptor phosphorylation recovered by the end of the dosing period for both SU11248 and SU6668, we conclude that substantial inhibition of the targets is not always required for agents targeting the VEGF and PDGF receptor pathways to achieve complete or near complete efficacy. If, however, substantial inhibition of the targets is not maintained for at least half the dosing period, as is observed at the 20 mg/kg dose of SU11248, there is a reduction in efficacy. Not surprisingly, when there is only a transient inhibition of the targets, such as is seen at the 5 mg/kg doses of SU11248, efficacy is very poor.

On the basis of the two primary findings from this study, we would predict that in patients, doses of SU11248 sufficient to produce plasma concentrations of 50–100 ng/ml for at least 12 h of a 24-h dosing interval would lead to inhibition of the target receptors sufficient to result in biological activity. Conversely, if we achieve and sustain substantially higher plasma concentrations in patients and show inhibition of target phosphorylation in the absence of biological activity, we would need to question the role of the targets in the pathophysiology of the disease. We recognize that the shape of the plasma concentration-time curve of SU11248 is likely to vary somewhat between species. However, the plasma level required to inhibit target RTKs and the duration of inhibition required to achieve anti tumor efficacy are independent of the shape of this curve and should therefore be translatable into the clinic.

In considering the results of this study, it is important to recognize that conclusions related to the duration of inhibition required to achieve efficacy are specific to a compound targeting this process (angiogenesis) by inhibiting these receptors (VEGF and PDGF receptors). It is possible, and even likely, that there would be a different requirement for angiogenesis inhibitors targeting other receptors (Ref. 29; *e.g.,* Tie 2 or VEGF receptor alone). It is also possible that a different PK/PD relationship (*e.g.,* potentially reduced target plasma concentration and duration of inhibition) would be identified in models in which SU11248 has direct antitumor activity through inhibition of an RTK(s) expressed on the tumor cell and required for survival (*e.g.,* FLT3 or KIT on some leukemias). In this respect, the observation in the present study that a similar dose-response effect for SU11248 was seen in a variety of different xenograft models has raised our confidence that the PK/PD relationship identified is likely to be broadly applicable to a variety of tumor settings in which SU11248 is acting primarily to inhibit angiogenesis.

One key factor that has enabled us to perform these studies with SU11248 was the demonstration that this compound has predictable, dose-dependent pharmacokinetics, with dose-dependent changes in exposure evaluated based on either total

exposure (area under the plasma concentration-time curve) or maximum plasma concentrations. In addition, we have been able to demonstrate that SU11248 does not covalently associate with its target RTKs, and that the biological effects of SU11248 *in vivo* are evident only when inhibitor is present at active concentrations in the plasma. Consequently we have not had to pursue more elaborate studies to identify a PK/PD relationship based on C_{max} exposures or long lasting biological activity.

In summary, we have outlined in this report an approach of elucidating PK/PD relationships in preclinical models to determine target plasma concentrations and duration of target inhibition required to achieve efficacy to aid in the clinical development of targeted therapies. Although the studies reported here specifically identify a PK/PD relationship for the antiangiogenic activity of SU11248 mediated by selective inhibition of VEGF and PDGF receptors, this approach should be easily adapted to other targeted therapies as long as *in vivo* models and techniques to directly assess compound effect in these models are available. The ultimate validation of this approach will be based on how well the results of this study predict the target exposure (50–100 ng/ml for 12 h of a 24-h dosing interval) for SU11248, which is currently in Phase I clinical trials.

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